treatments of diseases that are impacted by the loss of epigenetic maintenance.

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Dcr1 Tracked Down

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RNAi is essential for pericentromeric heterochromatic formation in *S. pombe*, and although Dcr1, the initiator protein of this process, has been biochemically well described, its subcellular localization has remained elusive. In this issue of *Developmental Cell*, Emmerth et al. now show that Dcr1 is dynamically shuttling between nucleus and cytoplasm, adding new insight into the subcellular mechanics of RNAi.

RNA interference (RNAi) is a mechanism that uses small RNAs as specificity factors to regulate gene expression. One of the best-studied small RNA species is the class of small interfering RNAs (siRNA). siRNAs are processed from long dsRNA molecules by dicer, an evolutionary well-conserved RNaseIII-like ribonuclease, containing additional functionalities like a helicase domain and two RNA binding domains: PAZ (Piwi Argonaute Zwille) and dsRBD (double-strand RNA binding domain). Processed siRNAs are loaded into a cytoplasmic or a nuclear effector complex called RNA-induced silencing complex (RISC) or RNA induced transcriptional silencing complex (RITS) respectively. In these complexes, siRNAs are bound by an Argonaute protein. Depending on the homology between siRNA and target RNA and the type of Argonaute, the target sequence can be silenced in two different ways: through posttranscriptional gene silencing (PTGS), which acts directly on the target RNA itself by target cleavage or translational inhibition, or through chromatin dependent gene silencing (CDGS), in which chromatin of the chromosomal locus producing the homologous sequence is remodeled into a repressive state (Carthew and Sontheimer, 2009; Moazed, 2009).

An organism that has specialized its RNAi machinery to a great extent to direct chromatin modification is *Schizosaccharomyces pombe* (*S. pombe*). In *S. pombe*, the formation of pericentromeric heterochromatin is a layered process in which first euchromatic histone modifications are removed, and next, Clr4 methylates H3K9 that is subsequently bound by Swi6. Biochemical studies have identified two distinct complexes that are essential for this heterochromatin formation. The Argonaute protein Ago1, the tryptophan GW-motif-containing protein Tas3, and the chromodomain protein Chp1 make up the RITS complex, which physically interacts with outer centromeric repeat transcripts in an siRNA-dependent manner. The RNA-directed RNA polymerase complex (RDRC), which physically interacts with RITS and amplifies the siRNA signal, has three core components: Rdp1 (an RNA-directed RNA polymerase), Cid12 (a polyadenylation polymerase), and the predicted helicase Hrr1. The interactions among RITS, RDRC, and the centromeric repeats are Dcr1 dependent, and Dcr1 itself has been reported to interact with RDRC (Colmenares et al., 2007; Moazed, 2009; Motamedi et al., 2004; Verdel et al., 2004). Previous reports based on Dcr1 overexpression studies reported counter-intuitive cytoplasmic localization (Carmichael et al., 2006). In this issue of *Developmental Cell*, Emmerth and colleagues readdress the question of the subcellular Dcr1 localization (Emmerth et al., 2010).

First, live cell imaging was performed on moderately expressed Dcr1-GFP fusion protein, revealing a predominantly nuclear localization. Interestingly, the nuclear localization was not diffuse, but colocalized with the nuclear pores in granule-like structures on the inner side of the nuclear membrane. These structures do not co-localize with RITS components or with chromatin, implying that siRNA generation and target recognition could be physically separated events. Interestingly in *Arabidopsis thaliana* (*A. thaliana*) and in the animal germline-specific Piwi pathway, the processing of CDGS-associated

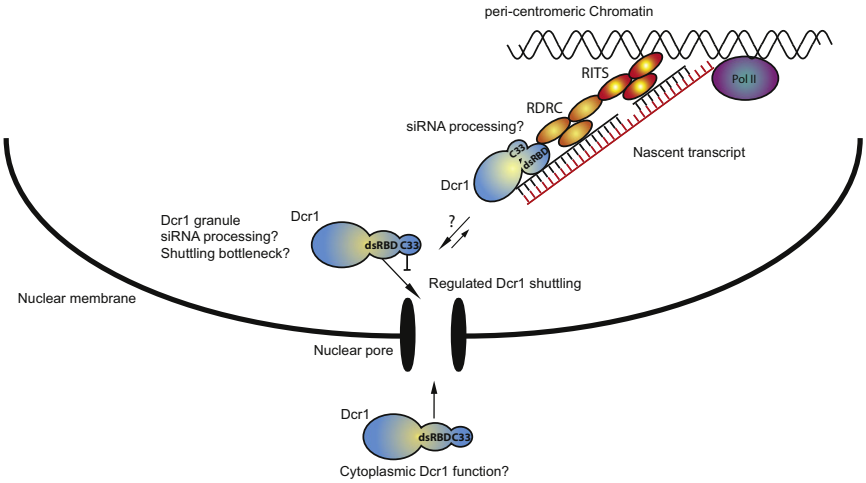

Figure 1. Schematic of Dcr1 Cytoplasmic-Nuclear Shuttling

RITS binds via siRNA-mediated base-pairing to pericentromeric nascent transcripts and recruits RDRC. Subsequently the nascent transcript is made double-stranded by the RDRC component Rdp1 and processed into siRNAs by Dcr1. Emmerth et al. (2010) show that Dcr1 shuttles between nucleus and cytoplasm. They also show that the dsRBD domain of Dcr1 stimulates this trafficking behavior, with the C33 domain inhibiting nuclear export. This may explain the observed accumulation of Dcr1 at the inside of the nuclear pores. Given the lack of *trans* RNAi activity and the fact that Rdp1 does not localize to pores, Dcr1-mediated siRNA processing likely occurs in association with chromatin and not at the nuclear pores, although this has not been addressed directly. It also remains unclear whether indeed RDRC-bound Dcr1 exchanges with nuclear pore-associated Dcr1.

small RNAs also occurs in distinct, granule-like nuclear structures, away from the chromatin target (Klattenhoff and Theurkauf, 2008; Li et al., 2006). Although so far no experiments have shown a clear mechanistic role for this separation, an argument could be that it would promote silencing in *trans*. However, in *S. pombe*, RNAi silencing appears to occur mainly in *cis*, and thus physical separation of processing and effector complexes would only introduce problems (Bühler et al., 2006). This, in combination with the fact that even the dsRNA-generating enzyme, Rdp1, does not colocalize with the nuclear pore-associated Dcr1 pool, suggests that the site of Dcr1 activity is in association with RDRC, and that the localization to the nuclear pore serves some other function.

To investigate the mechanism behind the observed Dcr1 localization, Emmerth and colleagues (2010) introduced C-terminal truncations in Dcr1, while retaining catalytic activity. Upon the removal of 33 C-terminal amino acids (Dcr1Δ33) Dcr1 became cytoplasmic, resulting in loss of pericentromeric siRNAs and silencing. An obvious first thought would be that this C33 domain of Dcr1 contains a nuclear localization signal (NLS), but delicate experiments show that this is not the case. On the contrary, even adding an NLS to Dcr1Δ33 under its endogenous promoter does not restore nuclear localization. Furthermore, fluorescence loss in photobleaching (FLIP) experiments on cells expressing Dcr1Δ33 indicate that Dcr1Δ33 shuttles between cytoplasm and nucleus and that this is mediated by the dsRBD domain of Dcr1 (Figure 1). Interestingly, nuclear localization of a larger C-terminal truncation of Dcr1 (Dcr1Δ103), also lacking the dsRBD domain, is restored upon adding a canonical NLS, indicating that the C33 domain somehow specifically blocks dsRBD-mediated nuclear export. Previous experiments suggested that the interaction between Dcr1 and Rdp1 required the C-terminal 103 amino acids (Colmenares et al., 2007), but from the data presented by Emmerth et al. (2010), it is now clear that this could be due to the cytoplasmic mislocalization of Dcr1Δ103. It would thus be interesting to see if nuclear localized Dcr1Δ103 would still interact with RDRC; the fact that nuclear Dcr1Δ103 rescues heterochromatin defects of Dcr1 mutant cells would suggest it does.

But why shuttle Dcr1 between cytoplasm and nucleus? And does the shuttling behavior relate to the Dcr1 granules at the nuclear pores? Interestingly, Emmerth et al. (2010) provide data suggesting that Dicer protein can be toxic to cells, in a way that is independent of its catalytic activity. Well-controlled Dicer localization could be one of the mechanisms to restrict unwanted Dicer activity, and one way to achieve that would be to impose a strong rate-limiting step at some point in a transportation cycle (Figure 1). The C33 domain of Dcr1 appears to do just that: inhibiting nuclear export of Dcr1 cycling between nucleus and cytoplasm, causing it to accumulate at the nuclear pores, on the inside of the nucleus. However, it should be noted that nuclear Dcr1Δ103 assembles into granules much like wild-type Dcr1, while still being toxic to cells (Emmerth et al., 2010), indicating that much more future work is required to fully understand Dcr1 dynamics and its potential regulation. Interestingly, of the four *A. thaliana* DICER-like proteins, the nuclear versions have an extended C-terminal tail, just like Dcr1, indicating that a similar localization mechanism could be at play. Such a conservation of Dicer shuttling behavior in plants and perhaps also in animal systems would have a big impact on our current thinking about small RNA biogenesis and would demand experiments aimed at unveiling nuclear functions of Dicer in animal cells.

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